**Citation:**

Arvidsson E, Viguerie N, Andersson I, Verdich C, Langin D, Arner P. Effects of different hypocaloric diets on protein secretion from adipose tissue of obese women. *Diabetes.* 2004 Aug;53(8):1966-71.

**PubMed ID:** 15277374

**Study Design:**

Randomized Clinical Trial

**Class:**

A - Click here for explanation of classification scheme.

**Research Design and Implementation Rating:**

POSITIVE: See Research Design and Implementation Criteria Checklist below.

**Research Purpose:**

To study the release of leptin, adiponectin, IL-6 and -8, TNFα, and PAI-1 in subcutaneous adipose tissue of 40 obese women before and after 10 weeks on moderate hypoenergetic diets with either low-fat/high-carbohydrate content or moderate-fat/moderate-carbohydrate content.

**Inclusion Criteria:**

- All subjects were part of a European multi-center study named Nutrient-Gene Interactions in Human Obesity:Implications for Dietary Guidelines, which examines the interaction between hypoenergetic diets and genes
- Obese women with a BMI of 30.9 - 47.7 kg/$m^2$
- Aged 21 - 49 years
- Otherwise healthy and free of medication

**Exclusion Criteria:**

None specifically mentioned.

**Description of Study Protocol:**

**Recruitment**

All subjects participated in a European multicenter study called Nutrient-Gene Interactions in Human Obesity: Implications for Dietary Guidelines.

**Design:** Randomized clinical trial

**Blinding used (if applicable):** implied with measurements

**Intervention (if applicable)**

- 40 subjects were randomly assigned to either a moderate-fat/moderate-carbohydrate (n=20) or a low-fat/high-carbohydrate (n=20) diet by the coordinating center in Copenhagen
- During the 10-week intervention, subjects visited or had telephone contact with the dietitian every week.
- Dietary target for the low-fat diet was 20-25% energy from fat, 15-20% protein, 60-65% carbohydrates
- Dietary target for the moderate-fat diet was 40-45% energy from fat, 15-20% protein, 40-45% carbohydrates
- Subjects performed 3-day weighed food record (2 weekdays and 1 weekend day) before starting the dietary intervention and at the end of the 10 week diet to estimate their habitual diet and compliance with the diet

- 1 day food records were completed in the 2nd, 5th and 7th week of the intervention
- Before and after the intervention, subjects had measurements done after an overnight fast: biopsy of subcutaneous adipose tissue, venous blood samples, weight, waist/hip circumference and bioimpedance were taken.
- Subjects were weighed every 2nd week when they visited the center

**Statistical Analysis**

- Data was compared between and within the 2 groups by:
  - Mann-Whitney U test,
  - Wilcoxon's signed-rank test,
  - Spearman's correlation,
  - Repeated measures ANOVA
- Variables were analyzed for normality and non-normally distributed variables were log transformed where appropriate
- Significance was set at $P \leq 0.05$

**Data Collection Summary:**

**Timing of Measurements**

- Subjects performed 3-day weighed food record (2 weekdays and 1 weekend day) before starting the dietary intervention and at the end of the 10 week diet
- 1 day food records were completed in the 2nd, 5th and 7th week of the intervention
- Before and after the intervention, subjects had measurements done after an overnight fast: biopsy of subcutaneous adipose tissue, venous blood samples, weight, waist/hip circumference and bioimpedance were taken.
- Subjects were weighed every 2nd week when they visited the center

**Dependent Variables**

- Body Mass Index
- Weight
- Waist-to-hip ratio
- Body Fat % - bioimpedance
- Glucose and insulin measured through fasting venous blood samples
- Fat cell volume, leptin, TNF-alpha, IL-6, IL-8, adiponectin and PAI-1 measured through biopsy

**Independent Variables**

- During the 10-week intervention, subjects visited or had telephone contact with the dietitian every week.
- Dietary target for the low-fat diet was 20-25% energy from fat, 15-20% protein, 60-65% carbohydrates
- Dietary target for the moderate-fat diet was 40-45% energy from fat, 15-20% protein, 40-45% carbohydrates
- Subjects performed 3-day weighed food record (2 weekdays and 1 weekend day) before starting the dietary intervention and at the end of the 10 week diet to estimate their habitual diet and compliance with the diet

**Control Variables**

**Description of Actual Data Sample:**

**Initial N**: 40 women

**Attrition (final N):**

- all 40 subjects completed the intervention, 20 in each group
- because of assay failures, the total number of subjects in each analysis was between 34 and 40

**Age**: 21-49 years

**Ethnicity**: not described

**Other relevant demographics**:

- none had undergone surgical treatment for their obesity
- 5 women (4 in the moderate-fat group and one in the low-fat group) were post-menopausal

**Anthropometrics:** no statistical differences between the groups at baseline in terms of age, resting energy expenditure, anthropometric measurements, blood pressure, fat cell volume, or fasting plasma levels of glucose, insulin, triglyceride and cholesterol.

**Location**:Stockholm, Sweden

**Summary of Results:**

**Key Findings**

- Total amount of calories from fat during the intervention was significantly different from baseline (P<0.01 for both groups)
- There was a marked difference in the percentage of total calories from carbohydrate and fat between the groups and also in the amount of dietary fiber (P<0.001 for all these factors)
- Percentage of calories from protein differed between the groups (P<0.01)
- The average weight reduction for all subjects was 7.7±0.4kg (7.5% decrease in body weight) and there was a continuous weight loss in both groups (P<0.0001)
- When both groups were considered together, leptin decreased significantly by ~20% (P=0.0004)
- Leptin secretion decreased by ~40% for all subjects put together and in both dietary groups (P≤0.005)
- Secretion of TNF-α and IL-6 and -8 decreased by 20-30% when all subjects were considered together (P≤0.02)
- Significant changes were found for IL-8 in both groups (P=0.040 for the moderate-fat group and 0.010 for the low-fat group), for TNF-α in the low-fat diet group (P=0.030) and for IL-6 in the moderate-fat diet group (P=0.040)
- There was a 20-35% decrease of the serum levels of leptin in the entire group of subjects as well as in both groups after the diet. In Spearman's correlation analysis, this decrease correlated with the change in leptin secretion (P=0.022)
- There was a 40% decrease in plasma PAI-1 activity in all subjects and in both groups after the diet (P≤0.002) but no difference between the groups (P=0.094)

| | Moderate Fat (n=20) | | | Low Fat (n=20) | | |
|---|---|---|---|---|---|---|
| | Before | After | P-value | Before | After | P-Value |
| Age (years) | 35.3±2.0 | --- | --- | 35.1±1.6 | --- | --- |
| BMI (kg/m$^2$) | 37.6±1.1 | 34.9±1.1 | <0.0001 | 36.6±0.9 | 33.7±0.9 | <0.0001 |
| Weight (kg) | 103±3.3 | 95.4±3.1 | <0.0001 | 102±2.7 | 94.4±2.7 | <0.0001 |
| Waist-to-hip-ratio | 0.95±0.01 | 0.94±0.01 | 0.30 | 0.94±0.009 | 0.93±0.01 | 0.038 |
| Body fat (%) | 45.9±1.0 | 43.2±1.0 | 0.0001 | 44.5±1.0 | 42.1±1.2 | 0.0002 |

Values are means±SE (Wilcoxon's signed-rank test)

Messenger RNA levels of leptin, TNF-α, IL-6 and -8, adiponectin, and PAI-1 before and after the diet

| | Moderate Fat (n=17) | | | Low Fat (n=17) | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before | After | P-value | Before | After | P-value | Before | After | P-Value |
| Leptin ($10^{-3}$ AU) | 1.54±0.32 | 1.18±0.27 | 0.0003* | 1.99±0.34 | 1.68±0.29 | 0.076 | 1.77±0.23 | 1.43±0.20 | 0.0004* |
| TNF-α ($10^{-7}$ AU) | 3.42±0.30 | 3.19±0.29 | 0.49 | 3.02±0.20 | 3.11±0.41 | 0.69 | 3.22±0.18 | 3.15±0.25 | 0.47 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| IL-6 ($10^{-8}$ AU) | 6.07±0.94 | 3.69±0.39 | 0.013* | 4.89±0.63 | 3.36±0.45 | 0.028* | 5.48±0.57 | 3.53±0.30 | 0.0011* |
| IL-8 ($10^{-7}$ AU) | 5.42±1.1 | 5.16±0.81 | 0.83 | 4.10±0.57 | 3.95±0.56 | 0.80 | 4.76±0.63 | 4.56±0.50 | 0.50 |
| Adiponectin ($10^{-3}$ AU) | 3.31±0.44 | 3.03±0.34 | 0.18 | 2.85±0.48 | 2.67±0.47 | 0.46 | 3.08±0.32 | 2.85±0.29 | 0.15 |
| PAI-1 ($10^{-6}$ AU) | 3.74±0.63 | 4.26±1.4 | 0.23 | 2.42±0.38 | 2.33±0.55 | 0.46 | 3.08±0.38 | 3.29±0.76 | 0.16 |

- *Values are means ±SE (Wilcoxon's signed-rank test). Messenger RNA values are expressed relative to the reference gene 18S.*
- **Significance was set at P≤0.05*

Amounts of secreted proteins from 400mg of fresh adipose tissue before and after dietary intervention

| | Moderate Fat (n=20) | | | Low fat (n=20) | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before | After | P-Value | Before | After | P-Value | Before | After | P-Value |
| Leptin (ng) | 56.7±4.8 | 36.7±4.7 | 0.0051* | 61.3±7.2 | 33.3±4.3 | 0.0040* | 59.0±4.3 | 35.0±3.2 | <0.0001* |
| TNF-α (ng) | 0.88±0.16 | 0.65±0.10 | 0.35 | 1.42±0.21 | 0.95±0.18 | 0.030* | 1.15±0.14 | 0.80±0.10 | 0.023* |
| IL-6 (ng) | 10.7±1.1 | 8.2±1.1 | 0.040* | 14.6±2.8 | 10.0±1.8 | 0.062 | 12.7±1.5 | 9.1±1.1 | 0.0050* |
| IL-8 (ng) | 42.8±5.8 | 33.8±5.2 | 0.040* | 52.3±6.9 | 42.0±8.6 | 0.010* | 47.8±4.6 | 38.1±5.2 | 0.0010* |
| Adiponectin (µg) | 2.32±0.24 | 2.72±0.35 | 0.16 | 2.27±0.20 | 2.37±0.22 | 0.42 | 2.29±0.15 | 2.53±0.20 | 0.12 |
| PAI-1 (ng) | 18.4±2.2 | 17.6±4.3 | 0.26 | 20.6±3.3 | 22.6±5.8 | 0.94 | 19.5±2.0 | 20.1±3.6 | 0.39 |

- *Values are means ±SE (Wilcoxon's signed-rank test). The amount of secreted protein is given per $10^7$ cells/2 h*
- **Significance was set at P≤0.05*

Circulating levels of leptin, TNF-α, IL-6, and adiponectin and PAI-1 activity before and after the diet

| | Moderate Fat (n=20) | | | Low fat (n=20) | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before | After | P-Value | Before | After | P-Value | Before | After | P-Value |
| Serum Leptin (ng/ml) | 38.8±3.1 | 29.9±2.6 | 0.0080* | 36.5±4.0 | 24.4±2.5 | 0.0012* | 37.6±2.5 | 27.2±1.8 | <0.0001* |
| Plasma TNF-α (pg/ml) | 4.80±1.6 | 4.84±1.5 | 0.65 | 2.79±0.72 | 2.16±0.53 | 0.059 | 3.79±0.87 | 3.50±0.79 | 0.56 |
| Plasma IL-6 (pg/ml) | 3.82±0.41 | 3.54±0.48 | 0.064 | 3.56±0.22 | 3.38±0.37 | 0.31 | 3.69±0.23 | 3.46±0.30 | 0.046* |
| Serum Adiponectin (µg/ml) | 16.4±1.7 | 16.9±1.6 | 0.88 | 18.3±1.8 | 20.6±1.9 | 0.30 | 17.3±1.3 | 18.7±1.3 | 0.40 |
| Plasma PAI-1 (IU/ml) | 28.6±4.1 | 19.4±4.1 | 0.0016* | 27.0±4.1 | 14.2±3.1 | <0.0001* | 27.8±2.9 | 16.8±2.6 | <0.0001* |

- *Values are means ±SE (Wilcoxon's signed-rank test).*
- **Significance was set at P≤0.05*

**Author Conclusion:**

In summary, this study suggests that energy supply per se and not the macronutrient composition is of importance for the regulation of the protein secretory function and gene expression of human adipose tissue, at least during energy restriction. Leptin is most sensitive for energy restriction, followed by cytokines and chemokines, whereas PAI-1 and adiponectin are not affected by a moderate weight decrease/reduction in energy intake. However, other sources for PAI-1 besides subcutaneous adipose tissue are very sensitive to nutritional changes.

***Reviewer Comments:***

*Study only 10 weeks long. Due to assay failures, total number of subjects in each analysis was between 34 and 40, leading to small numbers of subjects in groups.*

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***Research Design and Implementation Criteria Checklist: Primary Research***

| | | |
|---|---|---|
| **Relevance Questions** | | |
| 1. | Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies) | Yes |
| 2. | Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about? | Yes |
| 3. | Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice? | Yes |
| 4. | Is the intervention or procedure feasible? (NA for some epidemiological studies) | Yes |
| **Validity Questions** | | |
| **1.** | **Was the research question clearly stated?** | Yes |
| 1.1. | Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified? | Yes |
| 1.2. | Was (were) the outcome(s) [dependent variable(s)] clearly indicated? | Yes |
| 1.3. | Were the target population and setting specified? | Yes |
| **2.** | **Was the selection of study subjects/patients free from bias?** | Yes |
| 2.1. | Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? | Yes |
| 2.2. | Were criteria applied equally to all study groups? | Yes |
| 2.3. | Were health, demographics, and other characteristics of subjects described? | Yes |
| 2.4. | Were the subjects/patients a representative sample of the relevant population? | ??? |
| **3.** | **Were study groups comparable?** | Yes |

| | | |
|---|---|---|
| 3.1. | Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT) | ??? |
| 3.2. | Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline? | Yes |
| 3.3. | Were concurrent controls used? (Concurrent preferred over historical controls.) | Yes |
| 3.4. | If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis? | N/A |
| 3.5. | If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.) | N/A |
| 3.6. | If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")? | N/A |
| **4.** | **Was method of handling withdrawals described?** | Yes |
| 4.1. | Were follow-up methods described and the same for all groups? | Yes |
| 4.2. | Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.) | Yes |
| 4.3. | Were all enrolled subjects/patients (in the original sample) accounted for? | Yes |
| 4.4. | Were reasons for withdrawals similar across groups? | N/A |
| 4.5. | If diagnostic test, was decision to perform reference test not dependent on results of test under study? | N/A |
| **5.** | **Was blinding used to prevent introduction of bias?** | Yes |
| 5.1. | In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate? | N/A |
| 5.2. | Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.) | Yes |
| 5.3. | In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded? | N/A |
| 5.4. | In case control study, was case definition explicit and case ascertainment not influenced by exposure status? | N/A |
| 5.5. | In diagnostic study, were test results blinded to patient history and other test results? | N/A |
| **6.** | **Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?** | Yes |
| 6.1. | In RCT or other intervention trial, were protocols described for all regimens studied? | Yes |
| 6.2. | In observational study, were interventions, study settings, and clinicians/provider described? | N/A |
| 6.3. | Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect? | Yes |

| | | |
|---|---|---|
| 6.4. | Was the amount of exposure and, if relevant, subject/patient compliance measured? | Yes |
| 6.5. | Were co-interventions (e.g., ancillary treatments, other therapies) described? | Yes |
| 6.6. | Were extra or unplanned treatments described? | No |
| 6.7. | Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups? | Yes |
| 6.8. | In diagnostic study, were details of test administration and replication sufficient? | N/A |
| **7.** | **Were outcomes clearly defined and the measurements valid and reliable?** | Yes |
| 7.1. | Were primary and secondary endpoints described and relevant to the question? | Yes |
| 7.2. | Were nutrition measures appropriate to question and outcomes of concern? | Yes |
| 7.3. | Was the period of follow-up long enough for important outcome(s) to occur? | Yes |
| 7.4. | Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures? | Yes |
| 7.5. | Was the measurement of effect at an appropriate level of precision? | Yes |
| 7.6. | Were other factors accounted for (measured) that could affect outcomes? | Yes |
| 7.7. | Were the measurements conducted consistently across groups? | Yes |
| **8.** | **Was the statistical analysis appropriate for the study design and type of outcome indicators?** | Yes |
| 8.1. | Were statistical analyses adequately described and the results reported appropriately? | Yes |
| 8.2. | Were correct statistical tests used and assumptions of test not violated? | Yes |
| 8.3. | Were statistics reported with levels of significance and/or confidence intervals? | Yes |
| 8.4. | Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)? | Yes |
| 8.5. | Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)? | Yes |
| 8.6. | Was clinical significance as well as statistical significance reported? | Yes |
| 8.7. | If negative findings, was a power calculation reported to address type 2 error? | N/A |
| **9.** | **Are conclusions supported by results with biases and limitations taken into consideration?** | Yes |
| 9.1. | Is there a discussion of findings? | Yes |
| 9.2. | Are biases and study limitations identified and discussed? | Yes |
| **10.** | **Is bias due to study's funding or sponsorship unlikely?** | Yes |
| 10.1. | Were sources of funding and investigators' affiliations described? | Yes |
| 10.2. | Was the study free from apparent conflict of interest? | Yes |